Journal of the American Mosquito Control Association, 19(2):166–169, 2003

SCIENTIFIC NOTE

COMPOSITION AND ADULT ACTIVITY OF SALT-MARSH MOSQUITOES ATTRACTED TO 1-OCTEN-3-OL, CARBON DIOXIDE, AND LIGHT IN TOPSAIL ISLAND, NORTH CAROLINA[1]

LEOPOLDO M. RUEDA[2] AND ROBERT C. GARDNER[3]

ABSTRACT. By monitoring weekly for 3 months with Centers for Disease Control (CDC) light traps baited with carbon dioxide (CO_2) and light, 12 species of mosquitoes were collected from salt-marsh areas in Topsail Island, North Carolina: *Aedes vexans, Anopheles atropos, An. bradleyi, An. crucians, An. punctipennis, Culex pipiens, Cx. restuans, Cx. salinarius, Ochlerotatus sollicitans, Oc. taeniorhynchus, Oc. infirmatus,* and *Uranotaenia sapphirina.* The hourly activities of common salt-marsh mosquitoes, namely *Oc. sollicitans, Oc. taeniorhynchus, An. atropos, An. bradleyi,* and *Cx. salinarius,* were observed from 1700 to 0800 h by using a collection bottle rotator trap baited with 1-octen-3-ol (octenol), CO_2, and light. The mosquitoes exhibited different peaks of adult activity, with a significantly greater number of mosquitoes collected from 0600 to 0800 h than from 1700 to 1900 h.

KEY WORDS Mosquitoes, salt marsh, octenol, *Anopheles, Ochlerotatus, Aedes,* North Carolina, Culicidae

Salt-marsh mosquitoes are a nuisance along the coastal areas of North Carolina and other eastern states. During peak season, they cause severe annoyance to humans, and thus may affect the tourism industry along the coastal areas. These mosquitoes also might be important in the epidemiology of eastern equine encephalomyelitis virus, West Nile virus, and other arboviruses (Rueda et al. 2001). Little is known about the ecology of these mosquitoes in eastern North Carolina. Knowledge of the composition and biting habits of mosquitoes associated with salt-marsh areas is essential in establishing sound vector control programs. Furthermore, personal protection measures such as repellents, tent bednets, and wearing of long-sleeved clothes, require information on biting and feeding behavior of salt-marsh mosquitoes. In this study, we determined the composition and hourly activity pattern of salt-marsh mosquitoes in Topsail Island, North Carolina.

Mosquito collections were made in a salt-marsh area with the predominant plant species consisting of salt grass (*Distichlis spicata* L.), smooth cord grass (*Spartina alterniflora* Loisel.), and black needle grass (*Juncus roemerianus* Scheele) in the northern part of Topsail Island (34°27′N, 77°30′W), Onslow County, North Carolina. Margins of the salt marsh support dominant species such as marsh elder (*Iva imbricata* Walt.), wax myrtle (*Myrica cerifera* L.), yellow jasmine (*Gelsemium sempervirens* L.), and sea myrtle (*Baccharis frutescens* L.). The study area was described previously by Rueda et al. (2001).

To establish species composition, mosquitoes were collected weekly from July to September 1999 (1 night/wk for 10 wk; 2 traps/night) from 2 sites, about 2 km apart, by using traps baited with carbon dioxide (CO_2) and light as attractants. Each trap had an incandescent CM-47 light bulb (John Hock Co., Gainesville, FL). The CO_2 was from a 10-kg compressed gas cylinder. The average CO_2 release rate was calculated to be 57 g/h. The CO_2 tank was on the ground level, connected to a 2-m-long rubber hose, which vented near the trap air intake. Each site had 1 model 512, Centers for Disease Control (CDC)-type, 6-V, battery-powered trap (John Hock Co.) suspended from a piece of wood about 1.3 m above ground level. Mosquitoes near the intake were sucked into the trap and blown into a 1,000-ml plastic container. Traps were set every afternoon between 1600 and 1700 h, and the collections were picked up the following morning between 0800 and 0830 h.

To establish levels of hourly activity, mosquitoes were collected for 3 consecutive nights on September 13–16, 2000, and for 3 consecutive nights in October 3–6, 2000. A model 1512 collection bottle rotator–type, 12-V DC, battery-powered trap (John Hock), was placed on top of 2 closely standing parallel metal poles, about 1.3 m above ground level. Each trap was equipped with an attractant combination of 1-octen-3-ol (octenol), CO_2, and light. The octenol (Biosensory, Inc., Willimantic, CT) was a patented waxlike medium that releases 1.5 mg/h at 27°C. It was packaged in a crush-resistant plastic housing containing 3 g of octenol. The CO_2 was from a 2-liter plastic thermos containing 1 kg of dry ice. The average CO_2 release rate was cal-

[1] The views of the authors do not purport to reflect the views of the supporting agency.

[2] Walter Reed Biosystematics Unit, Department of Entomology, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500.

[3] Mosquito Control Section, Onslow County Public Works, 1222 Onslow Pines Road, Jacksonville, NC 28540.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2003		**00-00-2003 to 00-00-2003**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Composition and Adult Activity of Salt-Marsh Mosquitoes Attracted to 1-Octen-3-OL, Carbon Dioxide, and Light in Topsail Island, North Carolina	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Walter Reed Army Institute of Research,Department of Entomology,Silver Spring,MD,20910**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
see report

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**4**	

Table 1. Relative abundance of collected mosquito species in a Centers for Disease Control light trap baited with carbon dioxide and light in a salt marsh, Topsail Island, NC.

	Relative abundance (%)			3-month mean[1]	Total collected (*n*)
	July	Aug.	Sept.		
Aedes vexans	0.03	0.00	0.03	0.45	9
Anopheles atropos	6.32	9.40	16.93	258.35	5,167
Anopheles bradleyi	2.89	3.07	5.93	92.95	1,859
Anopheles crucians	0.00	0.00	0.67	6.80	136
Anopheles punctipennis	2.49	1.83	2.41	48.25	965
Culex pipiens	0.01	0.00	0.00	0.05	1
Culex restuans	0.00	0.01	0.00	0.05	1
Culex salinarius	6.16	1.98	17.19	218.85	4,377
Ochlerotatus infirmatus	0.01	0.00	0.76	7.75	155
Ochlerotatus sollicitans	33.35	30.66	25.73	613.55	12,271
Ochlerotatus taeniorhynchus	48.75	53.05	30.34	869.00	17,380
Uranotaenia sapphirina	0.00	0.00	0.01	0.05	1
Total collected (*n*)	10,936	11,127	20,259		

[1] Relative abundance means for 3 months are based on the total number of adults collected during the period.

culated to be 57 g/h. The CO_2 container and the octenol package were placed beside the trap near the air intake. The trap allowed segregation of the adult mosquito catch into 8 plastic bottles every hour by using a programmable timer. It had an internal back-up battery that maintained the date/time and switching program for several days without external power. Two traps (about 5 m apart) were used each night, with the 1st trap running from 1600 to 2400 h and the 2nd trap from 2400 to 0800 h. Each bottle of the trap, labeled with time and date of collection, had a dichlorvos mini-strip (BioQuip, Gardena, CA) to prevent the escape of collected mosquitoes.

Trap collection containers or bottles were removed each morning of the collection period. Specimens were removed, taken to the laboratory, sorted and identified by using Slaff and Apperson (1989), and totaled. Monthly abundance and comparisons of the hourly activities among mosquito species were analyzed with analysis of variance (SAS Institute 1985).

During 20 trap-nights in the salt marsh, 42,322 mosquitoes (12 species in 5 genera) were collected (Table 1). *Ochlerotatus taeniorhynchus* (Wiedemann), *Oc. sollicitans* (Walker), *Anopheles atropos* Dyar and Knab, and *Culex salinarius* Coquillett, respectively, were the 1st, 2nd, 3rd, and 4th most abundant species collected. Among the *Anopheles, An. atropos* was the dominant species, followed by *An. bradleyi* King and *An. punctipennis* (Say). Only 9 specimens of *Aedes vexans* Meigen and 1 specimen each of *Culex pipiens* L., *Cx. restuans* Theobald, and *Uranotaenia sapphirina* (Osten Sacken) were collected throughout the 3-month period of trapping. Overall, more specimens of common *Anopheles* and *Ochlerotatus* were collected in September than in July or August ($P \leq 0.05$).

The hourly adult activity is shown in Fig. 1 for 5 common species (*Oc. taeniorhynchus, Oc. sollicitans, An. atropos, An. bradleyi,* and *Cx. salinarius*), collected in a collection bottle rotator light trap baited with octenol, CO_2, and light. *Ochlerotatus taeniorhynchus* (27% of 6,938 total collected specimens) and *An. atropos* (33% of 889) had their significantly highest peak of activity from 0100 to 0200 h ($P \leq 0.05$). *Ochlerotatus sollicitans* (20% of 905) was most active at 0100 h. *Anopheles bradleyi* was highly active from 0400 to 0600 h, with its highest peak at 0500 h (22% of 2,271), whereas *Cx. salinarius* was at its peak at 2200 h (20% of 1,050). Both *Anopheles* species (82% of *An. atropos* collected; 69% of *An. bradleyi*) were more active from 0100 to 0600 h compared with other hours ($P \leq 0.05$). Other species collected from the rotator light trap included *Oc. infirmatus* Dyar and Knab (3 specimens each at 0500 and 0700 h), and *Ae. vexans, Ur. sapphirina,* and *Cx. restuans* (with 1 specimen each collected at 0700 h).

The species composition in our study was similar to that reported by Rueda et al. (2001), who used traps baited with CO_2 and light, except for *An. punctipennis* and *Cx. pipiens.* Based on the mean of collected females per trap night from July to September 1999, we found *Oc. taeniorhynchus* as the most abundant species, in contrast to being the 4th dominant species during the October 1998 collection (Rueda et al. 2001) when using traps baited with CO_2 and light in Topsail Island. Further studies should be done for at least a few years to verify the monthly fluctuation of mosquito populations in the salt-marsh area.

Numerous studies on the biting activity of mosquitoes (i.e., *Anopheles, Aedes,* and *Culex* species) have been conducted in various parts of the world. They include those conducted in Thailand (Rattanarithikul et al. 1996) and Malaysia (Hassan et al. 2001). Similar to the report of Carpenter and LaCasse (1955), we observed that the females of *Oc. taeniorhynchus* and *Oc. sollicitans* are persis-

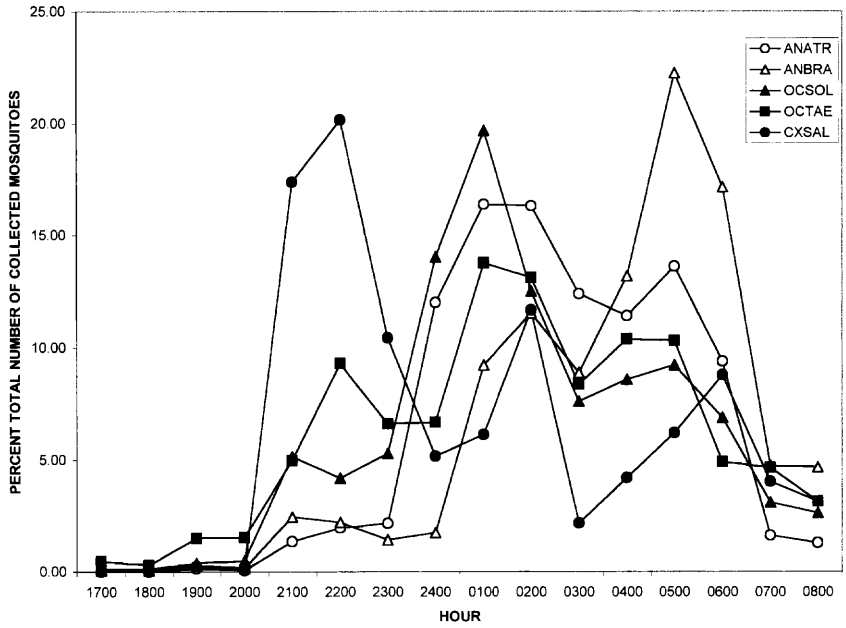

Fig. 1. Hourly activity of 5 commonly collected mosquito species in model 1512 collection bottle rotator–type trap baited with octenol, carbon dioxide, and light in a salt marsh, Topsail Island, NC (ANATR, *An. atropos*; ANBRA, *An. bradleyi*; OCSOL, *Oc. sollicitans*; OCTAE, *Oc. taeniorhynchus*; CXSAL, *Cx. salinarius*).

tent biters and may attack anytime during the day or night. Ebsary and Crans (1977) observed that the maximum activity of *Oc. sollicitans* in New Jersey occurred within 15–30 min of civil twilight at dusk and dawn. They noted that the total number of female *Oc. sollicitans* collected from the dawn period was significantly greater than for the dusk period. In our study, we found *Oc. sollicitans* throughout the collection period (1700–0800 h), with increased activity from 2100 to 0600 h and a peak at 0100 h. When we pooled the data, we also found that for *Oc. sollicitans, Oc. taeniorhynchus, An. atropos, An. bradleyi,* and *Cx. salinarius,* the number of females collected was significantly greater from 0500 to 0700 h (about dawn period) compared with those collected from 1700 to 1900 h (about dusk period) ($P \leq 0.05$).

In addition to obtaining information on species composition and adult activity, the larval breeding habitats of salt-marsh mosquitoes should be identified in order to develop effective mosquito control strategies. During our preliminary survey of Topsail Island (unpublished data), we collected the larvae of common species of *Anopheles, Ochlerotatus,* and *Culex* from salt-marsh habitats with the following water parameters: salinity 2.9–28.1 ppt (mean = 13.1 ppt, SE = 2.2 ppt), conductivity 5.2–46.1 mS/cm (mean = 38.8 mS/cm, SE = 17.1 mS/cm), pH 6.2–7.2 (mean = 6.6, SE = 0.3), and water temperature 16.0–31.4°C (mean = 22.5°C, SE = 4.2°C). The wide range of values of the water parameters may indicate the diverse nature of habitats and biting activity of the salt-marsh mosquitoes. Further studies should be done to ascertain the specific habitats of each mosquito species, including their water quality parameters and other environmental factors.

Biting mosquito composition and human contact by these species are important factors in determining nuisance level and possible disease transmission. People are likely to be motivated to use personal protection and other control methods when biting mosquito densities are high. The hourly activity of biting adult mosquitoes may be important in the epidemiology of viral and other human infectious diseases in the local areas.

We wish to thank A. Bangi and R. Brahm (College of Forestry, North Carolina State University, Raleigh, NC) for identifying plant species. Special thanks to N. H. Newton (Public Health Pest Management, North Carolina Department of Environment and Natural Resources) for giving us encouragement and support, and to R. C. Wilkerson, J. E. Pecor, and B. P. Rueda for reviewing the manuscript.

REFERENCES CITED

Carpenter SJ, LaCasse WJ. 1955. *Mosquitoes of North America (north of Mexico)* Berkeley, CA: Univ. Calif. Press.

Ebsary BA, Crans WJ. 1977. The biting activity of *Aedes sollicitans* in New Jersey. *Mosq News* 37:721–724.

Hassan AA, Rahman WA, Rashid MZA, Shahrem MR, Adanan CR. 2001. Composition and biting activity of *Anopheles* (Diptera: Culicidae) attracted to human bait

in a malaria endemic village in peninsular Malaysia near the Thailand border. *J Vector Ecol* 26:70–75.

Rattanarithikul R, Konishi E, Linthicum KJ. 1996. Observations on nocturnal biting activity and host preference of *Anopheles* collected in southern Thailand. *J Am Mosq Control Assoc* 12:52–57.

Rueda LM, Harrison BA, Brown JS, Whitt PB, Harrison RL, Gardner RC. 2001. Evaluation of 1-octen-3-ol, carbon dioxide, and light as attractants for mosquitoes associated with two distinct habitats in North Carolina. *J Am Mosq Control Assoc* 17:61–66.

SAS Institute. 1985. *SAS user's guide: statistics* Cary, NC: SAS Institute.

Slaff M, Apperson CS. 1989. A key to the mosquitoes of North Carolina and the mid-Atlantic states. *N C State Univ Agric Ext Serv Publ AG* 412:1–38.